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MICROSCALE TOPOGRAPHY OF DRAGLINE SILK OF *Nephila pilipes* (Fabricius, 1793)

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ABSTRACT

Spider can produce high-performance polymer in the form of silk. In the present work, atomic force microscopy was used as a qualitative measurement tool for determining the microscale topology and a quantitative tool for the surface nanostructure of dragline silk of *Nephila pilipes.* AFM study confirmed the existence of well organized bundles of nanofibrils in the dragline silk fibers. Bundles of fibrils into fibers enhance mechanical properties of silk threads. Roughness analysis of dragline silk suggested that this biomaterial has high toughness that may be suitable for dissipating high amounts of mechanical energy.

Key words: *Nephila pilipes*, dragline silk, AFM

INTRODUCTION

Orb weaving spiders spin significantly stronger and tougher dragline silk on average compared to other taxa, which may reflect the importance of the radial threads of orb webs in stopping the tremendous kinetic energy of flying insect prey (Agnarsson *et al.*, 2010). Due to its desirable properties, spider silk is researched intensively to better understand the interplay between its structure and performance.

AFM is a nondestructive technique which can provide rich topographic images of the silk fiber. The micrometer and nanometer scales affect different aspects of cell behavior and different cell type react differently to different surface topography (Zinger *et al.*, 2004); hence, it is important to study the surface roughness over the range of length scales.

MATERIALS AND METHODS

Dragline silk samples were taken from the frame thread of the orb web of *Nephila pilipes*. Silk threads were fixed on the thin glass slide with the help of adhesive transparent tape on both ends. Then it was mounted on piston with the help of double sided sticking tape. Piston was fixed on sample holder. Atomic force microscope, Nanoscope E, model no. 245 was used for imaging silk samples. Two dimensional deflection images of dragline silk were recorded in contact mode. Image analysis was performed with WSxM Nanoscope image processing software.

OBSERVATIONS AND RESULTS

Atomic force microscopy (AFM) was used to obtain topographic images of silk sample and to measure surface roughness of spider silk. Figure 1 shows representative surface topographies from AFM images of *Nephila pilipes* silk samples. The arrow direction indicates the fiber axis. These images were also used for calculation of surface roughness values of silk.

AFM images showed superimposed nanostructures on silk surface having darker and lighter color (Figure 1. d & f). Low regions are dark colored and higher regions are of lighter colored. The nanofibrillar nature of dragline silk of *Nephila pilipes* is clearly visible (Figure 1. a & c). The structural characteristic by stretching the dragline silk just below its typical BER was studied. This image shows the accordion-like pleats (Figure 1. e). For roughness analysis, three values were measured: the root mean square (RMS), the arithmetic average height (Ra) and the maximum height of hills (H). RMS represents the standard deviation of the height values within the given area and allows the surface roughness to be determined by statistical methods. Ra is the most frequently used roughness parameter. Roughness values changed with the scan size (Table - 1). Hence, the measurements were performed using different scan windows.

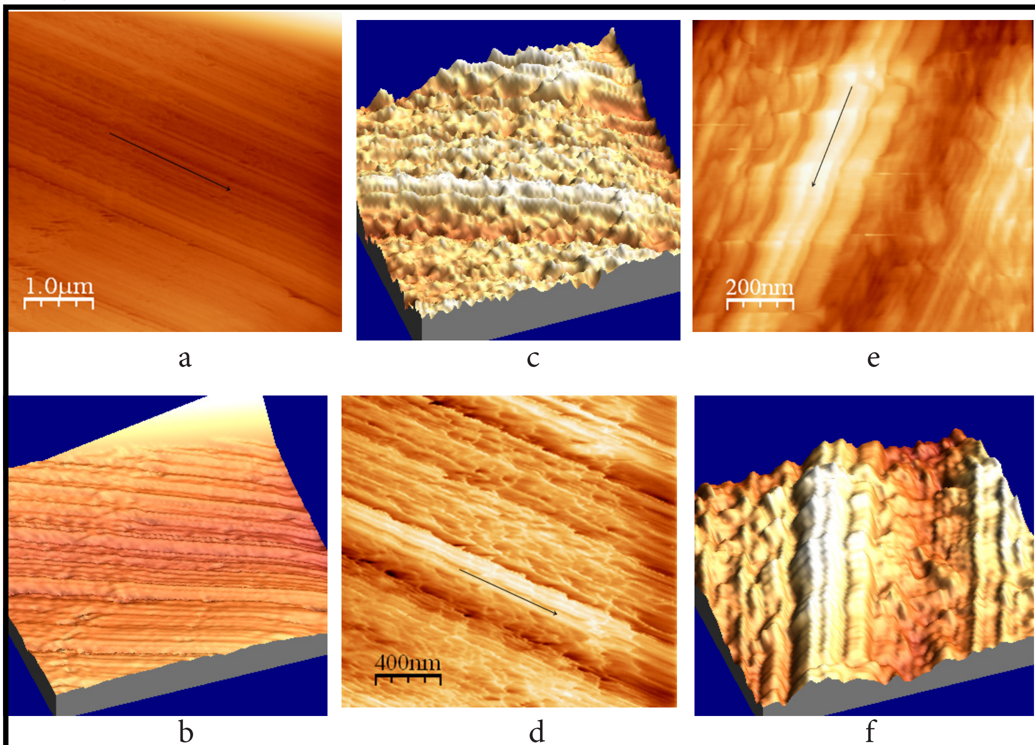

Figure 1: AFM images showing topography of dragline silk of *Nephila pilipes*. The arrow direction indicates the fiber axis. a. 2D image scan size 5 x 5 µm2 b. 3D image scan size 5 x 5 µm2 c. 2D image scan size 2 x 2 µm2; d. 3D image scan size 2 x 2 µm2; e. 2D image scan size 1 x 1 µm2; f. 3D image scan size 1 x 1 µm.2

Table -1: Surface roughness RMS, Ra and H for dragline silk of *Nephila pilipes*

Scan Size	R.M.S.(nm)	Ra(nm)	H(nm)
5 x 5 µm2	83.38	287.96	832.75
2 x 2 µm2	20.51	79.41	41.92
1 x 1 µm2	16.63	55.49	106.35

DISCUSSION

The characterization of material surface roughness on different length scale is important because biocompatibility of material is dependent on material chemistry and physical features as well as on surface roughness (Huang *et al.*, 2004).

AFM was used to provide topographic images and roughness for dragline silk of *Nephila pilipes*. AFM study confirmed the existence of well organized bundles of nanofibrils in the dragline silk fibers. Bundles of fibrils into fibers enhance mechanical properties of silk threads. A similar multifibrillar arrangement was identified in the silk fibers of insects cocoon silk and suggested that the multifibrillar structure of silk fibers contribute to its toughness by allowing dissipation energy in the controlled propagation of cracks (Hakimi *et al*., 2006).The peculiar topographical structure of spider dragline silk may be due to presence of secondary structural elements like β-sheets, β-turns, α-helices etc. These structures give semicrystalline nature to spider silk.

The outstanding mechanical properties of the major ampullate silk of *Nephila pilipes* are partly due to breaking extension ratio of about 1.2-1.4. Therefore, the structural characteristics that permit this range of extension by stretching the silk to just below its typical BER were studied. This image (Figure 1. e) suggests the clue to the mechanism that helps spider silk to achieve the optimal BER. The accordion-like pleats extend when silk is stretched. Whether this mechanism is the only mechanism for elasticity awaits a more detailed study of the correlation of fiber structures with incremental fiber extensions. However, these results do show that any molecular mechanism proposed to explain the BER must take into consideration the formation of supramolecular pleats (Li *et al*., 1994). They also described a structural model for spider dragline silk with the highly organized pleated fibrils accounts for both the elastic properties and mechanical strength of dragline silk. Figure 1. e shows close resemblance with structural model of Li, *et al*. (1994).

In AFM, dark areas depict low features and white areas depict high features. Therefore, the white portion from the figure is the projection from base matrix (Rajeev *et al*., 2001). Each spider silk probe shows a somewhat different structure. Fibers of the same species do not necessarily exhibit the same surface features (Schäfer *et al*., 2008).

CONCLUSION

AFM images show that silk fibers from *Nephila pilipes* are composed of bundle of nanofibrils running mostly parallel to each other and long axis of silk. The packing density of nanofibrills and surface roughness values of dragline silk of *Nephila pilipes* indicates the fact that, it is designed for extreme toughness.

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